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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/EP91/01718 (22) International Filing Date: 10 September 1991 (10.09.91) (30) Priority data: 21443 A/90 12 September 1990 (12.09.90) IT (71) Applicant (for all designated States except US): DEPHA TEAM S.R.L. [IT/IT]; Via Cassanese, 224, Palazzo Tiepolo, I-20090 Segrate (IT). (72) Inventors; and (75) Inventors/Applicants (for US only): PELLICCIARI, Roberto [IT/IT]; GARZON, Aaron [IL/IT]; CLERICI, Carlo [IT/IT]; PALAZZI, Camillo, Maria, Francesco, Giulio [IT/IT]; Via Cassanese, 224, Palazzo Tiepolo, I-20090 Segrate (IT).</p>		<p>(74) Agent: MINOJA, Fabrizio; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122, Milano (IT). (81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL (European patent), NO, PL, RO, SD, SE (European patent), SN (OAPI patent), SU*, TD (OAPI patent), TG (OAPI patent), US.  Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: 5-AMINOSALICYLIC ACID DERIVATIVES FOR THE THERAPY OF CHRONIC INFLAMMATORY BOWEL DISEASES  (57) Abstract  5-Aminosalicylic acid derivatives acylated at the amino group with glutamic or aspartic acid and/or having the carboxy group involved in a peptide bond with the leucyl-prolyl residue are useful as pro-drugs of 5-aminosalicylic acid.</p>		

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Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

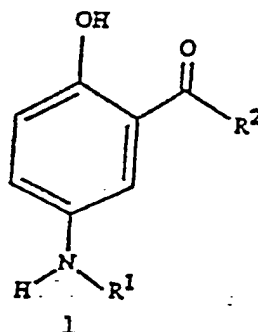
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5-AMINOSALICYLIC ACID DERIVATIVES FOR THE THERAPY OF  
CHRONIC INFLAMMATORY BOWEL DISEASES

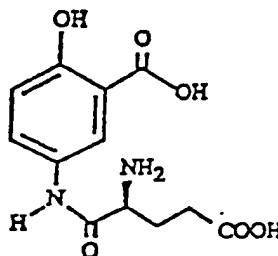
The invention refers to 5-aminosalicylic acid (5-ASA) derivatives of general formula 1



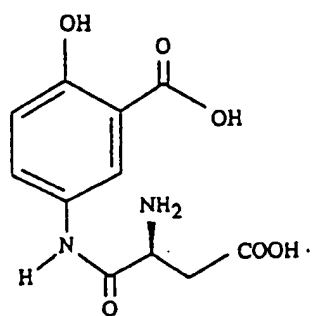
10 wherein  $R_1$  is hydrogen, glutamyl (Glu) or aspartyl (Asp) and  $R_2$  is OH or the residue leucyl-prolyl, with the proviso that  $R_1$  and  $R_2$  cannot be contemporaneously hydrogen and hydroxy, respectively.

15 The present invention also relates to a process for the preparation of compounds of formula 1.

The compounds of the invention of the general formula 1 have therefore the following formulae:

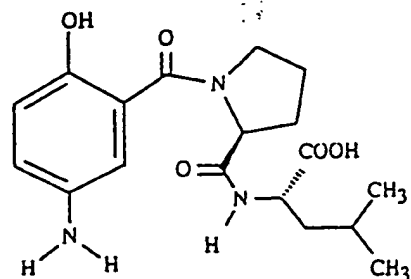


25 ( $R^1$ =Glu and  $R^2$ =OH)



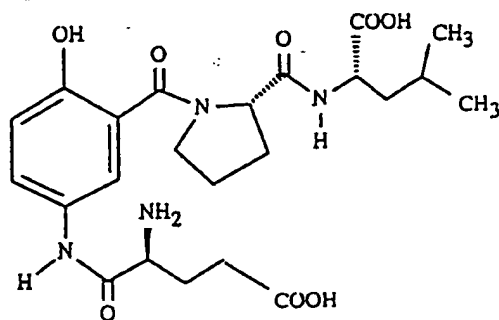
(R<sup>1</sup> = Asp and R<sup>2</sup> = OH)

3



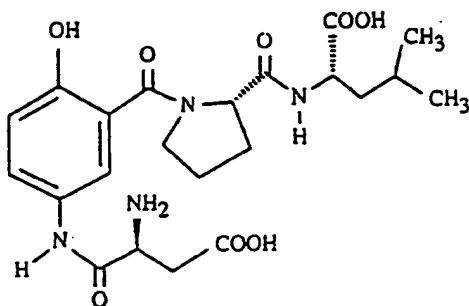
(R<sup>1</sup> = H and R<sup>2</sup> = Pro-Leu)

4



(R<sup>1</sup> = Glu and R<sup>2</sup> = Pro-Leu)

5



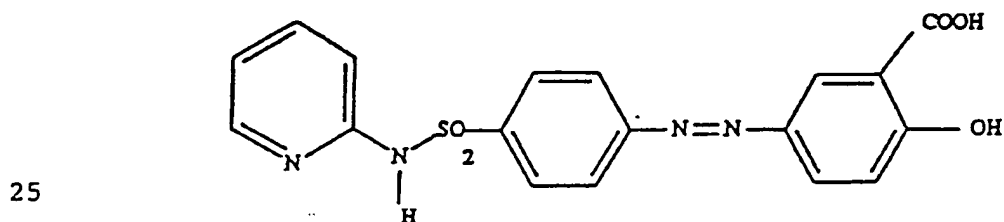
(R<sup>1</sup> = Asp and R<sup>2</sup> = Pro-Leu)

6

The invention refers also to the non-toxic salts of the above compounds, to pharmaceutical compositions containing them and to processes for the preparation thereof.

5 Said compounds are useful in chronic inflammatory bowel diseases, thanks to their topical antiinflammatory effect on intestinal mucosa portions affected by the lesions.

This activity involves interactions with specific  
10 peptidases present on the brush border of small intestine, which can hydrolyze selectively the amino acidic residues, releasing in situ the active principle 5-ASA. It is in fact known that some chronic  
15 inflammatory diseases, such as Chron's disease and ulcerative rectocolitis, are since many years treated with drugs able to inhibit the arachidonic acid derivatives biosynthesis, such as  $\text{PGE}_2$ , leucotrienes and thromboxane  $\text{B}_2$ . Sulfasalazine, of formula 7, was one of the first drugs used; it is metabolized to 5-ASA  
20 and sulfapyridine by reductive cleavage of the azide bond by intestinal bacteria.<sup>1,2</sup>



7

The properties of sulfasalazine seem to be non-therapeutic against Crohn's disease and of ulcerative  
30 rectocolitis. Moreover, sulfasalazine is responsible in some patients of the following side-effects:

- nausea and anorexia(dose-related)
- cutaneous rash and hematic dyscrasia (idiosyncratic phenomena)
- decrease of the number and of the motility of spermatozoa.

All the above effects are mainly due to sulfapyridine and in about 10% of the patients were so serious as to ask for the drop out of the treatment.<sup>3</sup>

Moreover 5-ASA, the active part of the sulfasalazine molecule, is not stable at the gastric pH and it is rapidly absorbed in the small intestine.<sup>4</sup> This prevents its use as such by the oral use, unless at high doses.

The compounds of general formula 1, having specific amino acidic residues bound to the 1-carboxy and/or 5-amino substituents of the corresponding 5-ASA, are hydrolyzed in vivo at the level of brush border of the ileum where specific aminopeptidases are present (Aminopeptidases A) 5-7,8,12 which are able to hydrolyze selectively an N-terminal amidic bond when a Glu or Asp residue is bound to that position. Moreover, dipeptides containing amino acids Glu or Asp as terminal residues are known to be resistant to pancreatic peptidases,<sup>5-7,8-10</sup> important requisite for the non-occurrence of the fast 5-ASA release. Finally, always in the brush border, a second class of peptidases is present, namely the carboxypeptidases, which are able to selectively hydrolyze a C-terminal amide bond between an amino acid and a penultimate Pro residue.<sup>1-3,4-6</sup>

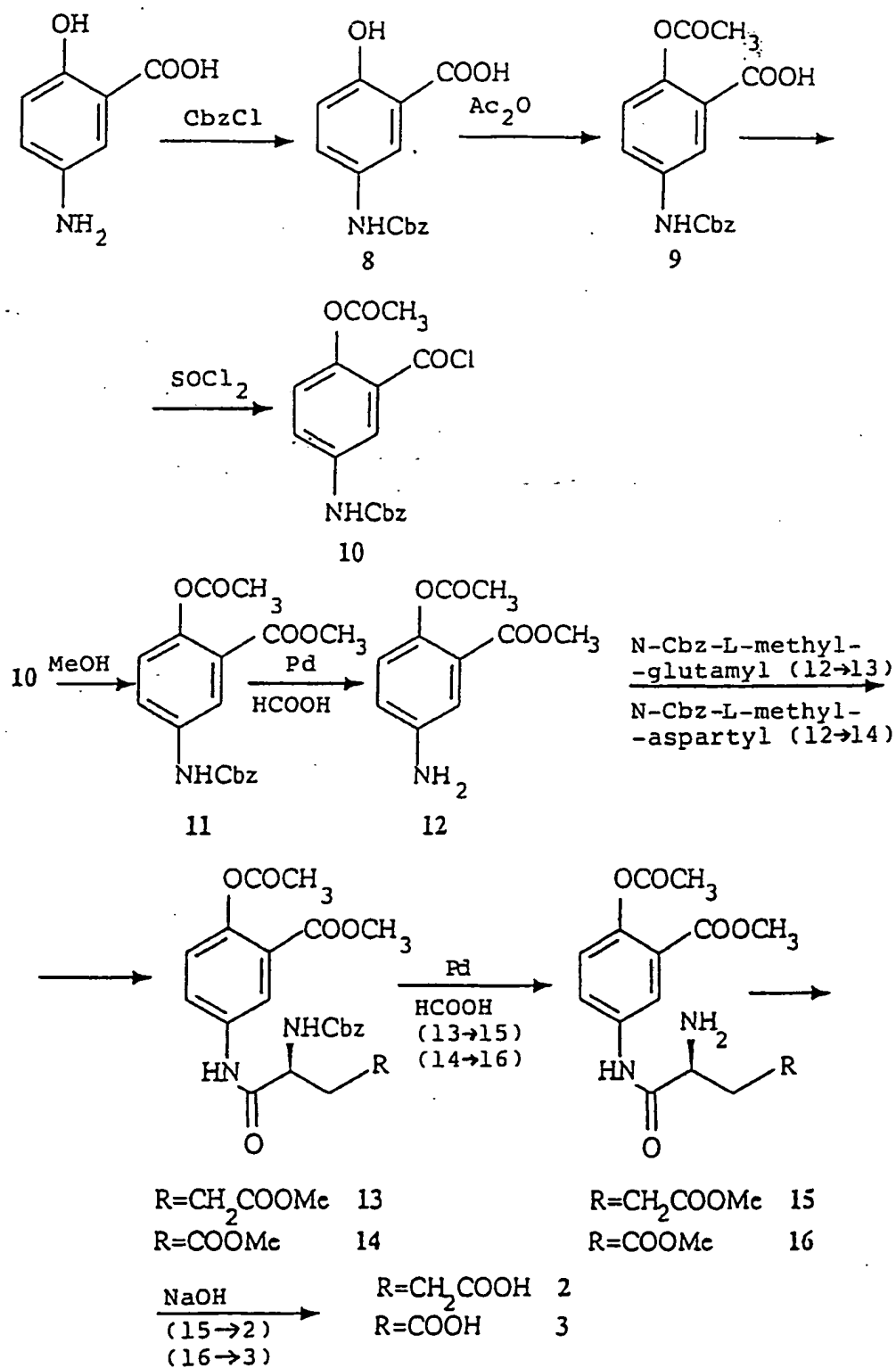
Thanks to these characteristics the compounds of

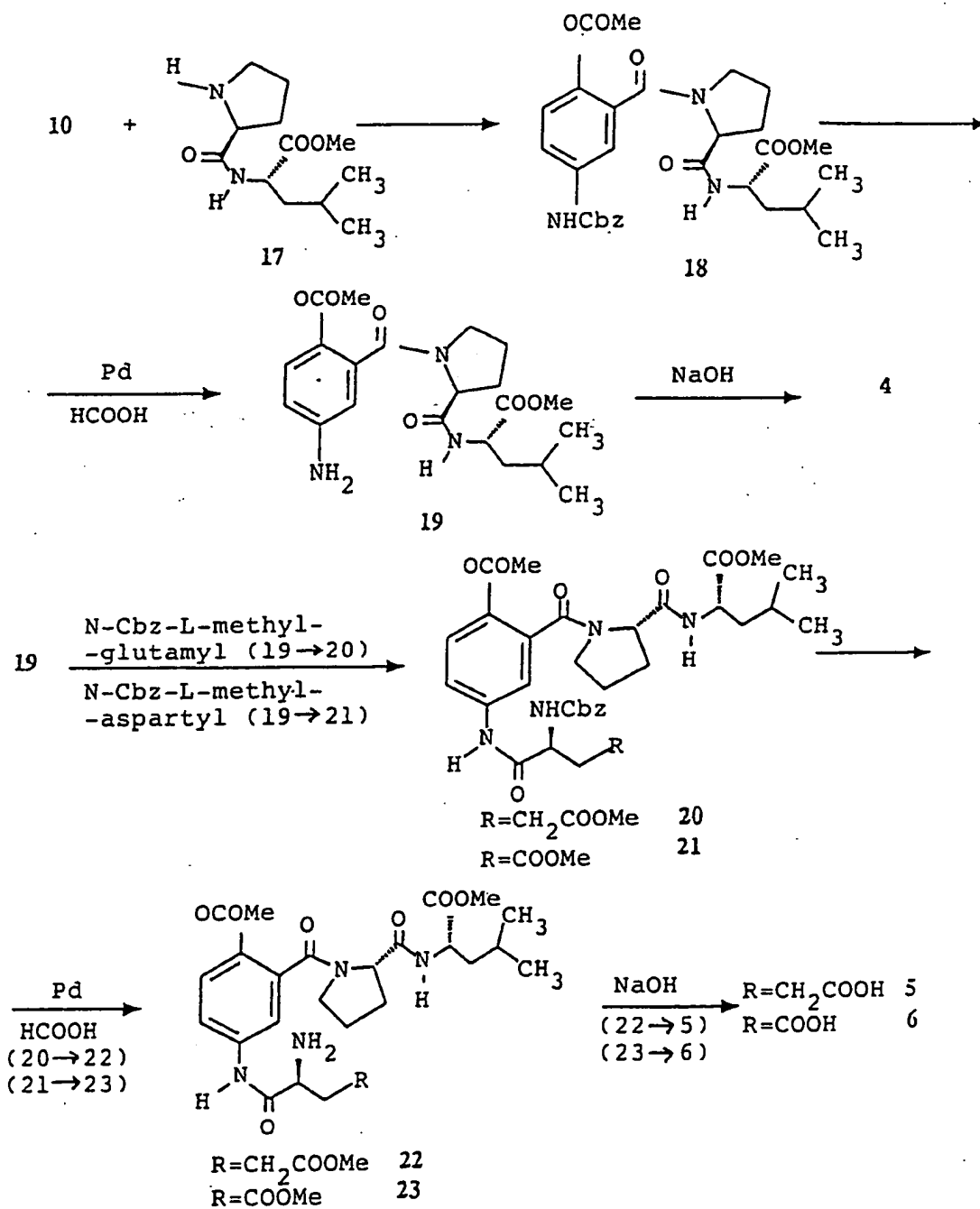
the invention may then undergo a chemoselective enzymatic hydrolysis so as to release 5-ASA directly at the distal intestine.

5 The compounds of the invention are easily prepared in liquid phase by means of usual methods for the peptide synthesis, starting from 5-aminosalicylic acid which is suitably protected and then reacted with an N-protected glutamic or aspartic acid derivatives and/or with a suitably protected leucyl-prolyl derivative. The  
10 removal of the protecting groups yields the desired compounds.

A general synthesis scheme is hereinbelow reported and the experimental conditions used for the preparation of the compounds 2, 3, 4, 5, 6 are  
15 described.







SUBSTITUTE SHEET

5-(N-benzyloxycarbonyl)-aminosalicylic acid 8

5-aminosalicylic acid (30 g, 0.20 mol) was suspended into a saturated  $\text{NaHCO}_3$  solution (500 ml). Solid  $\text{NaHCO}_3$  (10 g) was then added to the suspension at  $0^\circ\text{C}$  under stirring and then benzylchloroformate (36.7 g, 0.215 mol) was added dropwise.

An abundant precipitate of the desired product was formed when the reaction was over and it was filtered. The filtrate was washed with ethyl ether (2 x 100 ml), the aqueous phase was acidified with 10% HCl and extracted with ethyl acetate (3x100ml). The pooled organic extracts were dried on anhydrous sodium sulphate, evaporated under vacuum to give, together with the previously separated precipitate, 53 g of 8 (yield 92%).

$^1\text{H-NMR}$  ( $\text{DMSO}-d_6$ ):

ppm 5.17 (s, 2H,  $-\text{CH}_2\text{Ph}$ ); 7.00-8.05 (m, 8H, H-aromatic); 9.8 (s, 1H, COOH).

5-(N-benzyloxycarbonyl)-amino-2-acetylsalicylic acid 9

Pyridine ( 1.39 ml ) and acetic anhydride (35.5 g , 0.34 mol) were added to a stirred suspension of the compound 8 in acetic acid (280 ml). After 2 hours the formed precipitate was filtered and dried under vacuum. 40.1 g of 9 were obtained (yield 70%).

$^1\text{H-NMR}$  ( $\text{MeOH}-d_4$ ):

ppm 2.15 (s, 3H,  $-\text{CO}-\text{CH}_3$ ); 5.10 (s, 2H,  $-\text{CH}_2\text{Ph}$ ); 6.90-8.00 (m, 8H, H-aromatic).

5-(N-benzylcarbonyl)-amino-2-acetylsalicyl chloride 10

A suspension of 9 (35 g, 0.106 mol), thionyl chloride (25.2 g, 0.21 mol), pyridine (20 ml) in anhydrous benzene (150 ml), kept under stirring and in nitrogen

atmosphere, was refluxed for 3 hours. The resulting clear solution was cooled to obtain a white precipitate that was filtered and dried under vacuum to obtain 29.89 g of 10 (81% yield).

5 5-(N-benzyloxycarbonyl) amino-2-acetylsalicyl methyl ester 11

A suspension of 10 (10 g, 28.8 mmol) in methanol (100 ml) is kept under magnetic stirring until complete dissolution (about 2 hours). The resulting solution is  
10 evaporated under vacuum to give about 9.53 g of 11 (97% yield).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>):

ppm 2.2 (s, 3H, -CO-CH<sub>3</sub>); 3.8 (s, 3H, COOCH<sub>3</sub>); 5.10 (s, 2H, -CH<sub>2</sub>Ph); 6.8-7.9 (m, 8H, H-aromatic).

15 5-amino-2-acetylsalicyl methyl ester 12

A solution of 11 (7 g, 20.4 mmol) in methanol (100 ml) and formic acid (10 ml) is poured into a column containing Palladium Black. The obtained eluate is evaporated under reduced pressure. The residue is  
20 crystallized from ethyl acetate/n-hexane to give 3.58 g of 12 (yield 84%).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>):

ppm 2.25 (s, 3H, -CO-CH<sub>3</sub>); 3.7 (s, 3H, COOCH<sub>3</sub>); 7.3-7.8 (m, 3H, H-aromatic).

25 5-(N-benzyloxycarbonyl-L-methylglutamyl)-amino-2-acetylsalicyl methyl ester 13

5-(N-benzyloxycarbonyl-L-methylaspartyl)-amino-2-acetylsalicyl methyl ester 14

A solution of N-benzyloxycarbonyl-5-L-methylglutamyl)-  
30 (2.12 g, 7.18 mmol) or of N-benzyloxycarbonyl-4-L-methylaspartyl (2.01 g, 7.18 mmol) in anhydrous

methylene chloride (10 ml), kept at  $-10^{\circ}\text{C}$  under magnetic stirring and argon atmosphere, is added with N-methylmorpholine (1 ml, 7.9 mmol), then with isobutyl chloroformate (1 ml, 7.18 mmol). The reaction mixture is left to react for 30 minutes, then it is filtered and the filtrate is combined with a solution of 12 (7.15 mmol) in anhydrous methylene chloride (40 ml). After that, the reaction mixture is kept under magnetic stirring and argon atmosphere for 4 hours, then it is evaporated and the residue is chromatographed through silica gel column (d.- 6 cm, h.- 20 cm), eluting first with chloroform (500 ml), then with 99:1 chloroform/methanol. 3.04 g of 13 (87% yield) or 2.88 g of 14 (85% yield) are obtained.

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) of 13:

ppm 2.2 (s, 3H,  $-\text{CO}-\text{CH}_3$ ); 2.36 (m, 4H,  $-\text{CH}_2-\text{CH}_2-$ ); 3.7 (s, 3H,  $\text{COOCH}_3$ ); 3.73 (s, 3H,  $\text{COOCH}_3$ ); 4.35 (m, 1H,  $-\text{CH}-\text{NH}-$ ); 5.15 (s, 2h,  $-\text{CH}_2\text{Ph}$ ); 6.2 (d, 1H, NH); 7.15-8.05 (s, 3H, H-aromatic).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) of 14:

ppm 2.22 (s, 3H,  $-\text{CO}-\text{CH}_3$ ); 2.33 (m, 2H,  $-\text{CH}_2-$ ); 3.7 (s, 3H,  $\text{COOCH}_3$ ); 3.70 (s, 3H,  $\text{COOCH}_3$ ); 4.30 (m, 1H,  $-\text{CH}-\text{NH}-$ ); 5.15 (s, 2h,  $-\text{CH}_2\text{Ph}$ ); 6.2 (d, 1H, NH); 7.15-8.05 (s, 3H, H-aromatic).

5-(N-L-methylglutamyl)-amino-2-acetylsalicyl methyl ester 15

5-(N-L-methylaspartyl)-amino-2-acetylsalicyl methyl ester 16

A solution of 13 (5 g, 10.29 mmol) or 14 (4.85 g, 10.29 mmol) in methanol (50 ml) and formic acid (5 ml) is poured into a column containing Palladium Black. The

obtained eluate is evaporated under reduced pressure, then the residue is crystallized from ethyl acetate/n-hexane to give 3.44 g of 15 (95% yield) or 3.34 g of 16 (96% yield).

5 (N-L-glutamyl)-amino-2-salicylic acid 2

(N-L-aspartyl)-amino-2-salicylic acid 3

A solution of 15 (2 g, 5.68 mmol) or 16 (1.91 g, 5.68 mmol) in 2N NaOH (50 ml) is kept under magnetic stirring for 6 hours at room temperature. Then the  
10 mixture is acidified with 10% HCl to form a precipitate which is filtered and dried under vacuum, to give 1.5 g of 2 (94% yield) or 1.46 g of 3 (96% yield).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>) of 2:

ppm 2.35 (m, 4H, -CH<sub>2</sub>-CH<sub>2</sub>-); 4.30 (m, 1H, -CH-NH-); 6.1  
15 (d, 1H, NH); 7.2-8.05 (m, 3H, H-aromatic).

<sup>1</sup>H-NMR CDCl<sub>3</sub> of 3:

ppm 2.30 (m, 2H, -CH<sub>2</sub>-); 4.35 (m, 1H, -CH-NH-); 6.1 (d, 1H, NH); 7.2-8.05 (m, 3H, H-aromatic).

20 5-(N-benzyloxycarbonyl-amino-2-acetylsalicyl L-proline-L-leucine-O-methyl 18

A solution of 10 (28 g, 0.08 mol) and 17 (19.4 g, 0.08 mol) in anhydrous carbon tetrachloride (300 ml), kept under magnetic stirring and nitrogen atmosphere, is refluxed for 12 hours. Then the reaction mixture is  
25 filtered and the filtrate is evaporated under reduced pressure. The residue is chromatographed on silica gel (d.- 5 cm, h.- 20 cm), eluting with chloroform to obtain 25 g of the tripeptide 18 (57% yield).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>):

30 ppm 0.95 (2d, 6H, -CH(CH<sub>3</sub>)<sub>2</sub>); 1.5-2.15 (m, 10H, -CO-CH<sub>3</sub>, -CH<sub>2</sub>-CH, -CH<sub>2</sub>-CH<sub>2</sub>-CH-CO); 3.15 (s, 3H, COOMe); 3.7

(m, 2H,  $-\text{CH}_2-\text{N}-\text{CO}$ ); 4.2-4.7 (m, 2H,  $-\text{CH}-\text{CO}$ ,  $-\text{NH}-\text{CH}-\text{COOMe}$ ); 5.1 (d, 2H,  $\text{CH}_2\text{Ph}$ ); 6.9-8.0 (m, 8H, H-aromatic).

5-amino-2-acetylsalicyl L-proline-L-leucine-O-methyl 19

A solution of 18 (17 g, 0.03 mol) in methanol (110 ml) and formic acid (11 ml) is poured into a column containing Palladium Black. The obtained eluate is evaporated under reduced pressure, then the residue is dissolved with a  $\text{NaHCO}_3$  saturated solution (100 ml) and extracted with ethyl acetate. The combined organic extracts are dried over anhydrous sodium sulphate and evaporated under vacuum, to give 10 g of 19 (80% yield).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):

ppm 0.95 (2d, 6H,  $-\text{CH}(\text{CH}_3)_2$ ); 1.6-2.35 (m, 10H,  $-\text{CO}-\text{CH}_3$ ,  $-\text{CH}_2-\text{CH}$ ,  $-\text{CH}_2-\text{CH}_2-\text{CH}-\text{CO}$ ); 3.6-4.8 (m, 7H,  $\text{COOMe}$ ,  $-\text{CH}_2-\text{N}-\text{CO}$ ,  $-\text{CH}-\text{CO}$ ,  $-\text{NH}-\text{CH}-\text{COOMe}$ ); 6.85 (m, 3H, H-aromatic).

5-aminosalicyl L-proline-L-leucine 4

A solution of 19 (5 g, 0.012 mmol) in 2N  $\text{NaOH}$  (50 ml) is kept under magnetic stirring for 6 hours at room temperature, then the reaction mixture is neutralized with 10%  $\text{HCl}$  to form a precipitate which is then filtered and dried under vacuum to obtain 4.09 g of 4 (94% yield).

$^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):

ppm 0.95 (2d, 6H,  $-\text{CH}(\text{CH}_3)_2$ ); 1.6-2.30 (m, 7H,  $-\text{CH}_2-\text{CH}$ ,  $-\text{CH}_2-\text{CH}_2-\text{CH}-\text{CO}$ ); 4.2 (m, 4H,  $-\text{NH}-\text{CH}-\text{COOH}$ ,  $-\text{CH}_2-\text{N}-\text{CO}$ ,  $-\text{CH}-\text{CO}$ ); 6.80-7.20 (m, 3H, H-aromatic).

5-[N-(N'-benzyloxycarbonyl)-5'-methyl-L-glutamyl]-amino-2-acetylsalicyl L-proline-L-leucine-O-methyl 20

5-[N-(N'-benzyloxycarbonyl)-4'-methyl-L-aspartyl]-ami-

no-2-acetylsalicyl L-proline-L-leucine-O-methyl 21

A solution of N-benzyloxycarbonyl-5-L-methylglutamyl (2.12 g, 7.18 mmol) or of N-benzyloxycarbonyl-4-L-methylaspartyl (2.01 g, 7.18 mmol) in anhydrous methylene chloride (10 ml), kept at -10°C under magnetic stirring and argon atmosphere, is added with N-methylmorpholine (1 ml, 7.9 mmol), then with isobutyl chloroformate (1 ml, 7.18 mmol). The reaction mixture is left to react for 30 minutes, then it is filtered and the filtrate is combined with a solution of tripeptide 19 (3 g, 7.15 mmol) in anhydrous methylene chloride (8 ml). The reaction mixture is kept under magnetic stirring and argon atmosphere for 4 hours, then it is evaporated and the residue is chromatographed through silica gel column (d.- 3 cm, h.- 20 cm), eluting first with chloroform (500 ml), then with 99:1 chloroform/methanol. 3 g of tripeptide 20 (60% yield) or 3.18 g of 21 (65% yield) are obtained.

<sup>1</sup>H-NMR (CDCl<sub>3</sub>) of 20:

ppm 0.85 (2d, 6H, -CH(CH<sub>3</sub>)<sub>2</sub>); 1.35-2.50 (m, 14H, -CO-CH<sub>3</sub>, -CO-(CH<sub>2</sub>)<sub>2</sub>-CH, -CH<sub>2</sub>-CH, -CO-CH-CH<sub>2</sub>-CH<sub>2</sub>-); 3.5-4.1 (m, 8H, COOMe, COOMe, -CH<sub>2</sub>-N-CO); 4.15-4.75 (m, 3H, -CO-CH-NHCbz, -NH-CH-COOMe, -N-CH-CO); 5.1 (s, 2H, -CH<sub>2</sub>Ph); 6.2-7.8 (s, 8H, H-aromatic).

<sup>1</sup>H-NMR (CDCl<sub>3</sub>) of 21:

ppm 0.80 (2d, 6H, -CH(CH<sub>3</sub>)<sub>2</sub>); 1.35-2.50 (m, 12H, -CO-CH<sub>3</sub>, -CO-CH<sub>2</sub>-CH, -CH<sub>2</sub>-CH, -CO-CH-CH<sub>2</sub>-CH<sub>2</sub>-); 3.55-4.15 (m, 8H, COOMe, COOMe, -CH<sub>2</sub>-N-CO); 4.15-4.80 (m, 3H, -CO-CH-NHCbz, -NH-CH-COOMe, -N-CH-CO); 5.15 (s, 2H, -CH<sub>2</sub>Ph); 6.2-7.8 (s, 8H, H-aromatic).



5-(N-5'-methyl-L-glutamyl)-amino-2-acetylsalicyl L-proline-L-leucine-O-methyl 22

5-(N-4'-methyl-L-aspartyl)-amino-2-acetylsalicyl L-proline-L-leucine-O-methyl 23

5 A solution of 20 (3 g, 4.31 mmol) or 21 (2.93 g, 4.31 mmol) in methanol (50 ml) and formic acid (50 ml) is poured into a column containing Palladium Black. The obtained eluate is evaporated under reduced pressure, then the residue is dissolved with a NaHCO<sub>3</sub> saturated  
10 solution (50 ml) and extracted with ethyl acetate (4x20 ml). The combined organic extracts are dried over anhydrous sodium sulphate and evaporated under vacuum, to give 2.13 g of tripeptide 22 (88% yield) or 2.13 g of 23 (90% yield).

15 <sup>1</sup>H-NMR (CDCl<sub>3</sub>) of 22:

ppm 0.85 (2d, 6H, -CH(CH<sub>3</sub>)<sub>2</sub>); 1.37-2.40 (m, 14H, -CO-CH<sub>3</sub>, -CO-(CH<sub>2</sub>)<sub>2</sub>-CH, -CH<sub>2</sub>-CH, -CH<sub>2</sub>-CH<sub>2</sub>-CH-CO); 3.5-4.1 (m, 8H, COOMe, COOMe, -CH<sub>2</sub>-N-CO); 4.15-4.70 (m, 3H, -N-CH-CO, -NH-CH-COOMe, -CO-CH-NH<sub>2</sub>); 7.1-7.7 (s, 3H, H-aromatic).

20 <sup>1</sup>H-NMR (CDCl<sub>3</sub>) of 23:

ppm 0.87 (2d, 6H, -CH(CH<sub>3</sub>)<sub>2</sub>); 1.30-2.45 (m, 12H, -CO-CH<sub>3</sub>, -CO-CH<sub>2</sub>-CH, -CH<sub>2</sub>-CH, -CH<sub>2</sub>-CH<sub>2</sub>-CH-CO); 3.5-4.15 (m, 8H, COOMe, COOMe, -CH<sub>2</sub>-N-CO); 4.20-4.75 (m, 3H, -N-CH-CO, -NH-CH-COOMe, -CO-CH-NH<sub>2</sub>); 7.1-7.7 (s, 3H, H-aromatic).

25 5-(N-L-glutamyl)-aminosalicyl L-proline-L-leucine 5

5-(N-L-aspartyl)-aminosalicyl L-proline-L-leucine 6

A solution of 22 (2 g, 3.56 mmol) or 23 (1.95 g, 3.56  
30 mmol) in 2N NaOH (30 ml) is kept under magnetic stirring for 6 hours at room temperature, then the

reaction mixture is neutralized with 10% HCl and cooled to obtain a precipitate which is filtered and dried under vacuum to give 1.75 g of 5 (99% yield) or 1.62 g of 6 (95% yield).

5  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) of 5:

ppm 0.85 (2d, 6H,  $-\text{CH}(\text{CH}_3)_2$ ); 1.37-2.30 (m, 11H,  $-\text{CO}-(\text{CH}_2)_2-\text{CH}$ ,  $-\text{CH}_2-\text{CH}$ ,  $-\text{CH}_2-\text{CH}_2-\text{CH}-\text{CO}$ ); 3.8 (m, 2H,  $-\text{CH}_2-\text{N}-\text{CO}$ ); 4.15-4.70 (m, 3H,  $-\text{N}-\text{CH}-\text{CO}$ ,  $-\text{CH}-\text{COOH}$ ,  $-\text{CO}-\text{CH}-\text{NH}_2$ ); 7.1-7.6 (m, 3H, H-aromatic).

10  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) of 6:

ppm 0.85 (2d, 6H,  $-\text{CH}(\text{CH}_3)_2$ ); 1.35-2.35 (m, 9H,  $-\text{CO}-\text{CH}_2-\text{CH}$ ,  $-\text{CH}_2-\text{CH}$ ,  $-\text{CH}_2-\text{CH}_2-\text{CH}-\text{CO}$ ); 3.83 (m, 2H,  $-\text{CH}_2-\text{N}-\text{CO}$ ); 4.13-4.75 (m, 3H,  $-\text{N}-\text{CH}-\text{CO}$ ,  $-\text{CH}-\text{COOH}$ ,  $-\text{CO}-\text{CH}-\text{NH}_2$ ); 7.1-7.6 (m, 3H, H-aromatic).

15 The best pharmacokinetic characteristics of the compounds of the present invention can be evidenced analyzing the recovery urines and feces of 5-ASA and N-acetyl-5-ASA, compared with 5-ASA as such and sulfasalazine.

20 Particularly, male Fischer rats weighing about 200-250 g were used.

Part of the animals were subjected to outer colostomy. After general anaesthesia by means of pentobarbital (Nembutal 7.5 mg/100 g body weight)  
25 administered intraperitoneally, a median laparotomy was effected, then ascending colon was sectioned and connected to the outer abdominal wall, followed by suture of the incision.

After about 7 days, which were required to restore  
30 the intestinal function, 5-ASA, sulfasalazine and derivatives 2, 3, 4, 5 and 6 were administered at doses

equivalent to 60 mg/kg of 5-ASA through a metal probe inserted into stomach.

The same administration was carried out also in animals which had not been subjected to surgery.

5        The rats were then placed in metabolic cages, from which feces and urine were withdrawn at 2 hour intervals for 48 hours.

The test results are summarized in the following table.

10

	Urine	Feces
5-ASA	79±3%	05±3%
Sulfasalazine	30±7%	37±4%
15    Compound 2	34±4%	45±4%
Compound 3	33±3%	46±3%
Compound 4	53±3%	25±4%
Compound 5	15±6%	54±9%
20    Compound 6	14±4%	54±3%
Recovery percentages of 5-ASA + N-acetyl-5-ASA in feces and urine by HPLC		

25        The study evidences that the compounds of the present invention are particularly active when compared with sulfasalazine and 5-ASA.

Therefore, the compounds of the invention can conveniently be used as active ingredients of pharmaceutical compositions for the treatment of  
30    chronic inflammatory bowel diseases such as Crohn's disease and ulcerous rectocolitis, since they have a topical anti-inflammatory activity on the injured tracts of intestinal mucosa.

35        Examples of said pharmaceutical compositions for the oral administration comprise capsules, pearls,

tablets, sachets, containing 200 to 1,000 mg of the active ingredient per unitary dose, to be administered two/three times daily, according to the disease to be treated and the conditions of the patient.

5           Pharmaceutical compositions for the rectal administration are suppositories containing 200 to 1,000 mg of the active ingredient per unitary dose, to be administered two/three times daily, and clysters, containing 2 to 10 g of the active ingredient per  
10           unitary dose, to be administered one/two times daily, according to the disease to be treated and the conditions of the patient.

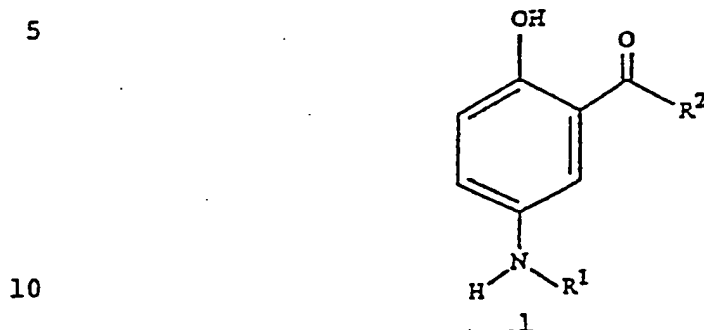
          The compositions of the invention may contain other active ingredients having a complementary or  
15           anyhow useful activity.

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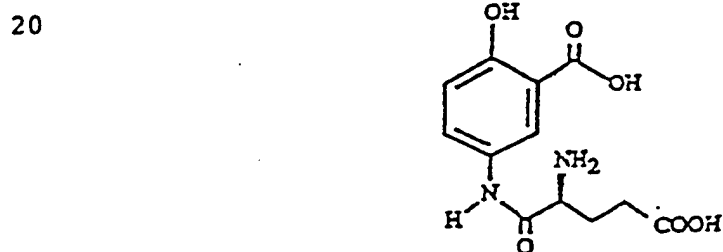
CLAIMS

1. Compounds of general formula 1

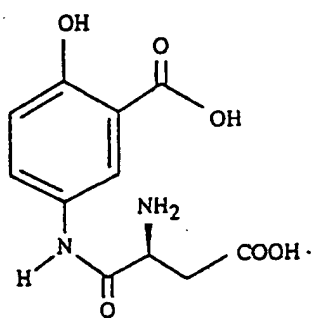


wherein  $R_1$  is hydrogen, glutamyl (Glu) or aspartyl (Asp) and  $R_2$  is OH or the residue leucyl-prolyl, with the proviso that  $R_1$  and  $R_2$  cannot be contemporaneously hydrogen and hydroxy, respectively.

2. A compound according to claim 1 having the following formulas 2-6.

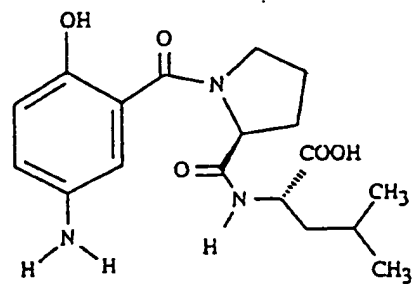


( $R^1$ =Glu and  $R^2$ =OH)



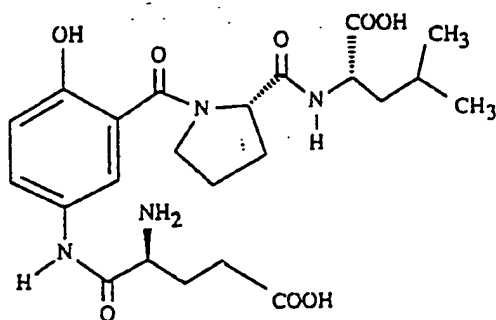
(R<sup>1</sup> = Asp and R<sup>2</sup> = OH)

3



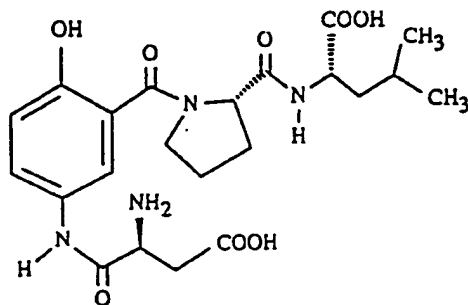
(R<sup>1</sup> = H and R<sup>2</sup> = Pro-Leu)

4



(R<sup>1</sup> = Glu and R<sup>2</sup> = Pro-Leu)

5



(R<sup>1</sup> = Asp and R<sup>2</sup> = Pro-Leu)

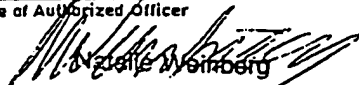
6

3. A process for the preparation of the compounds of claims 1-2 comprising the reaction of a suitably protected 5-aminosalicylic acid derivative with an N-protected glutamic or aspartic acid derivative and/or  
5 with a suitably protected leucyl-prolyl derivative followed by removal of the protecting groups.
4. Pharmaceutical compositions containing as the active principle a compound of claims 1-2 in admixture with a pharmaceutically acceptable carrier.
- 10 5. The use of the compounds of claims 1-2 for the preparation of medicaments for the therapy of chronic intestinal inflammations, crohn's disease and ulcerative colitis.



# INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/01718

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 07 K 5/02, A 61 K 37/02//A 61 K 31/60		
<b>II. FIELDS SEARCHED</b> Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC5	A 61 K; C 07 C; C 07 K	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	DE, B2, 2920292 (NITTO BOSEKI CO., LTD.) 22 May 1980, see the whole document --	1-5
A	WO, A1, 8603199 (ITALFARMACO S.P.A.) 5 June 1986, see the whole document --	1-5
A	WO, A1, 8102672 (FARMACEUTISK LABORATORIUM FERRING A/S) 1 October 1981, see the whole document --	1-5
<p>* Special categories of cited documents:<sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
11th December 1991		21.01.92
International Searching Authority  EUROPEAN PATENT OFFICE		Signature of Authorized Officer 

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	CH, A, 536278 (MERCK & CO.) 15 June 1973, see the whole document --	1-5
A	CH, A, 555805 (MERCK & CO.) 15 November 1974, see the whole document --	1-5
A	US, A, 4505898 (ROBERT E. MARKS ET AL.) 19 March 1985, see the whole document -----	1-5

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO. PCT/EP 91/01718**

SA 50946

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on 31/10/91  
The European Patent office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		GB-A-B- 2034690	11/06/80
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		SE-B-C- 445829	21/07/86
		SE-A- 7905009	17/05/80
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US-A- 4505898	19/03/85	US-A- 4452783	05/06/84

For more details about this annex : see Official Journal of the European patent Office, No. 12/82